

# EXHIBIT E

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(54) Title  
**DOMINANT AMYLOSE-EXTENDER MUTANT OF MAIZE**

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(71) Applicant(s)  
 IOWA STATE UNIVERSITY RESEARCH FOUNDATION, INC.

(72) Inventor(s)  
 DONALD S. ROBERTSON; PHILIP S. STINARD

(74) Attorney or Agent  
 CALLINAN LAWRIE, Private Bag 7, KEW VIC 3101

(57) We have isolated from our Mutator transposable element stock an ae mutant allele, Ae-5180, that is dominant, requiring the presence of only one dose of Ae-1580 in the endosperm to produce a kernel with the glassy-sugary phenotype characteristic of homozygous ae. It has been found that homozygous lines of Ae-5180, developed without selection for high amylose, can produce starch with an amylose content as high as 72%. Lines with one or two Ae-5180 alleles in the endosperm also have amylose contents of 70% (Dr. John Robyt, Iowa State University, personal communication). Therefore, this mutant is uniquely adaptable for breeding purposes because the triploid endosperm of maize produces the same high level of amylose whether Ae-5180 is present in one, two, or three copies. It is expected that selection of Ae-5180 lines for a higher amylose content could increase the percent amylose.

#### CLAIM

1. High amylose starch maize seeds containing at the *amylose-extender* (ae) locus at least one dominant mutant allele Ae-5180.
7. Viable maize seeds and succeeding generations thereof grown from the seeds deposited under ATCC Accession No. 40499, and maize seeds to which the Ae-5180 dominant mutant allele of said deposited seeds has been transferred and succeeding generations thereof, said maize seeds and succeeding generations thereof containing the dominant mutant allele Ae-5180 at their amylose-extender (ae) locus.

625542

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

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CONVENTION APPLICATION FOR A PATENT

We, IOWA STATE UNIVERSITY RESEARCH FOUNDATION, INC., a corporation organized under the laws of the State of Iowa, located at Iowa State University, Ames, Iowa, 50011, United States of America, hereby apply for the grant of a patent for an invention entitled "DOMINANT AMYLOSE-EXTENDER MUTANT OF MAIZE", which is described in the accompanying complete specification.

This application is a Convention Application and is based on the applications for a patent or similar protection made in the United States of America on 28 November, 1988 numbered 276882.

Our address for service is: Care of CALLINAN LAWRIE, Patent Attorneys, 278 High Street, Kew, Victoria, 3101, Australia.

D A T E D this 7th day of August 1991.

IOWA STATE UNIVERSITY RESEARCH FOUNDATION, INC.

By Their Patent Attorneys:

CALLINAN LAWRIE

To: The Commissioner of Patents



To: The Commissioner of Patents.

AUSTRALIA  
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## COMPLETE SPECIFICATION

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### TO BE COMPLETED BY APPLICANT

• Name of Applicant: IOWA STATE UNIVERSITY RESEARCH FOUNDATION, INC.

• Address of Applicant: Iowa State University, Ames, Iowa, 50011, United States of America

• Actual Inventors: Donald S. Robertson and Philip S. Stinard

Address for Service: CALLINAN LAWRIE, Patent and Trade Mark Attorneys, 278 High Street, Kew, 3101, Victoria, Australia

Complete Specification for the invention entitled: "DOMINANT AMYLOSE-EXTENDER MUTANT OF MAIZE"

The following statement is a full description of this invention, including the best method of performing it known to me:-

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## DOMINANT AMYLOSE-EXTENDER MUTANT OF MAIZE

## FIELD OF INVENTION

This invention relates to mutants of maize which produce high-amylose starch, which are called amylose-extender or ae mutants.

## 5 BACKGROUND OF INVENTION

Maize starch is composed of two glucose polymers: (1) amylose which is a straight chain molecule, and (2) amylopectin which is a branched chain molecule. Mutations at the amylose-extender, or ae, locus of maize result in an increase in the amylose content of the endosperm relative to its amylopectin content. For  
10 example, the increase may be from around 25% to as high as 70% (Shannon, J.C. and Garwood, D.L., 1984, Genetics and Physiology of Starch Development in Starch: Chemistry and Technology, 2nd edition, ed. Whistler, BeMiller, and Faschall, Academic Press, Inc. Orlando, pp. 25-86). Starch with high amylose content can be used to produce tough, edible or biodegradable films and gels.  
15 (Starch: Chemistry and Technology, 2nd edition, ed, Whistler, BeMiller, and Faschall, Academic Press, Inc., Orlando, 1984).

To date, all reported ae mutants have been simple recessives, requiring the mutants to be homozygous in order to produce starch with sufficiently high amylose content. (Zuber, M.S., Grogan, C.O., Deatherage, W.L., Hubbard, J.E.,  
20 Schulze,





W. E. and MacMasters, M. M., 1958. Breeding high amylose corn. Agronomy Journal 50:9-12. Vineyard, M. L., Bear, R. P., MacMasters, M. M. and Deatherage, W. L., 1958. Development of "Amylomaize"--corn hybrids with high amylose starch: I. Genetic considerations. Agronomy Journal 50:595-598. Helm, J. L., Ferguson, V. L. and Zuber, M. S., 1967. Development of high-amylose corn (Zea mays L.) by the backcross method. Crop Science 7:659-662. Garwood, D. L., Shannon, J. C. and Creach, R. G., 1976. Starches of endosperms, <sup>possessing</sup> ~~processing~~ different alleles at the amylose-extendor locus of Zea mays L. Cereal Chemistry 53:355-364.) The known recessive mutant alleles of the amylose-extendor locus have already proven useful in the production of high-amylose starches. The amylose content of starches from the best recessive ae hybrids currently available is around 70%. (Shannon, J. C. and Garwood, D. L., 1984. Genetics and Physiology of Starch Development in Starch: Chemistry and Technology, 2nd edition ed., <sup>Whistler, BeMiller and</sup> ~~B-E~~ Faschall, Academic Press Inc. Orlando. pp. 25-86.) Current techniques require the development of homozygous ae inbreds, followed by the production of hybrid seed for planting in open-pollinated production fields.

A dominant mutant allele at the ae locus, if available, could be used to accelerate the development of high-amylose inbred or hybrid lines because such a dominant genetic trait, which produces the high-amylose starch, expresses itself in every generation of crossing and thus is readily followed. The recessive ae mutant alleles are not as readily followed in a



crossing regime. A dominant mutant allele can be expected to have other uses and advantages.

#### SUMMARY OF THE INVENTION

5 According to a first embodiment of the invention there is provided high amylose starch maize seeds containing at the *amylose-extender* (ae) locus at least one dominant mutant allele Ae-5180.

According to a second embodiment of the invention there is provided high amylose maize seeds produced by crossing two maize lines, said seeds containing at the *amylose-extender* (ae) locus at least one dominant mutant allele Ae-5180.

10 According to a third embodiment of the invention there is provided high amylose starch maize seeds containing at the *amylose-extender* (ae) locus homozygous Ae-5180 alleles.

According to a fourth embodiment of the invention there is viable maize seeds and succeeding generations thereof grown from the seeds deposited under ATCC Accession No. 40499, and maize seeds to which the Ae-5180 dominant mutant allele of said deposited seeds has been transferred and succeeding generations thereof, said maize seeds and succeeding generations thereof containing the dominant mutant allele Ae-5180 at their amylose-extender (ae) locus.

20 According to a fifth embodiment of the invention there is provided the method of developing hybrid maize seeds containing high amylose starch, comprising crossing a first maize line with a second maize line, at least one of said lines containing at its *amylose-extender* (ae) locus at least one dominant mutant allele Ae-5180.

25 According to a sixth embodiment of the invention there is provided the method for directly producing high amylose maize seeds, comprising crossing a homozygous Ae-5180 line as a male onto a detasseled or male sterile wild type (Ae Ae) hybrid maize line.

30 We have isolated from our Mutator transposable element stock an ae mutant allele, Ae-5180, that is dominant, requiring the presence of only one dose of Ae-1580 in the endosperm to produce a kernel with the glassy-sugary phenotype characteristic of homozygous ae. It has been found that homozygous lines of Ae-5180, developed without selection for high amylose, can produce starch with an amylose content as high as 72%. Lines with one or two Ae-5180 alleles in the endosperm also have amylose contents of 70% (Dr. John Robyt, Iowa State University, personal communication). Therefore, this mutant is uniquely adaptable

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for breeding purposes because the triploid endosperm of maize produces the same high level of amylose whether Ae-5180 is present in one, two, or three copies. It is expected that selection of Ae-5180 lines for a higher amylose content could increase the percent amylose.

- 5 In addition to providing an improved means for production of high-amylose starch varieties of hybrid maize for users of high-amylose starch, it is expected that the dominant ae mutant of this invention will have other uses. Recessive ae alleles are known to alter the branching and chain length of kernel starches. They also interact in unique ways with other mutants that alter the structure of maize
- 10 starches, such as waxy, dull, and sugary (Vineyard, M.L., Bear, R.P., MacMasters, M.M., and Deatherage, W.L., 1958. Development of "Amylomaize"—Corn hybrids with high amylose starch: I. Genetic considerations. Agronomy Journal 50:595-598. Kramer, H.H., Whistler, R.L., and Anderson, E.G., 1956. A new gene interaction in the endosperm of maize. Agronomy Journal 48:170-172.) It may be possible to
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cross Ae-5180 into other mutant lines to tailor-make starches to suit special industrial uses. Hybrids between Ae-5180 and currently existing recessive ae inbreds may result in synergistic effects between the dominant and recessive alleles that could result in an increased amylose content of kernel starch, or result in alternations in starch quality which may have commercial value. Ae-5180 could be used to accelerate the development of high-amylose inbred and hybrid lines.

When Ae-5180 is crossed as a male to an inbred line, the  $F_1$  kernels express a mutant phenotype that is characteristic for a given inbred. If the amylose content of the  $F_1$  kernels is predictive of the amylose content of Ae-5180 after it has been converted to the inbred background, then promising high-amylose inbreds could be selected for further development after one generation of crosses. Likewise, it may be possible to select promising high-amylose hybrids by crossing Ae-5180 to different elite hybrids and analyzing the amylose content of the progeny kernels. Ae-5180 may allow the use of novel techniques for the production of high-amylose maize.

Current techniques using recessive mutant alleles require the development of homozygous ae inbreds, followed by the production of hybrid seed for planting in open-pollinated fields. High-amylose Ae-5180 material could also be produced in this manner. Alternatively, because Ae-5180 induces high amylose levels (70%) when in one or two copies in the endosperm, one could adapt systems of detasseling or cytoplasmic male sterility to produce female rows of wildtype starchy hybrids that could be pollinated by homozygous Ae-5180 male rows. This system could be used to take advantage of

currently available elite starchy hybrid lines and thereby tremendously increase the yield of high-amylose kernels.

#### DETAILED DISCLOSURE

In our 1984 Y1 WX gl8/Y1 WX Gl8 Mu isolation plot, a single glassy kernel was observed in a population of 388,688 kernels. We planted this kernel in 1985, and self-pollinated and outcrossed the resulting plant to a standard starchy line (Standard Q60). The self-pollinated ear segregated for a sugary kernel type in an approximate 1:2 ratio of starchy to sugary kernels. The outcross ear segregated in an approximate 2:1 ratio of starchy to sugary kernels. Thus, it appeared that our mutant (which we first designated Su\*-5180) was dominant, but was transmitted in a reduced frequency since the ratios observed differ significantly ( $p < 0.001$ ) from the expected values of 1:3 and 1:1 in the self and outcross respectively (Table 1).

Table 1. Counts of starchy and sugary kernels on the self and outcross ears of the original Ae-5180 mutant plant grown in 1985. The outcross was made to a standard starchy line.

Plant No.	Selfed ear			Male Outcross ear		
	Starchy	Sugary	% Sugary	Starchy	Sugary	% Sugary
5180-1	123	244	66.5	213	103	32.4
	1:3 Chi-square = 14.1916 ( $p < 0.001$ )			1:1 Chi-square = 39.4465 ( $p < 0.001$ )		

Further tests (described below) showed that Su\*-5180 is fully female transmissible, but frequently shows reduced

transmission through the male, when in competition with wildtype pollen, as is the situation in a heterozygous plant. We tested Su\*-5180 for allelism with su1, su2, du and ae, because these mutants have a sugary or glassy phenotype similar to that of Su\*-5180. Selfs of (Su\*-5180/su1), (Su\*-5180/su2), and (Su\*-5180/du) segregated for fully starchy kernels as well as the parental types and their presumed combinations (double mutants). Such results are expected if Su\*-5180 is not allelic to su1, su2, or du. The selfs of (Su\*-5180/ae), however, produced only sugary or glassy kernels, a result expected if Su\*-5180 is a dominant mutant allele of ae. Backcrosses of (Su\*-5180/ae) by ae ae stocks also produced ears with only sugary or glassy kernels, confirming the allelism of Su\*-5180 with ae. We now designate our dominant amylose-extender mutant Ae-5180.

The expression of Ae-5180 in our standard backgrounds varies from slightly shrunken and tarnished to wrinkled sugary to brittle. This variability occurs whether Ae-5180 is present in the endosperm in 1, 2, or 3 doses. We have made F1 reciprocal crosses of Ae-5180 to several different inbred lines, and observed that the phenotype of the F1 kernels seems to show a maternal effect. When our standard lines carrying Ae-5180 are used as the female parent in the inbred crosses, the F1 kernels exhibit the variability in phenotype noted above. However, when the inbred lines are used as females, the expression of Ae-5180 is consistent for a given inbred, but varies depending on which inbred is the female parent. The inbred B73 shows the most

extreme expression, the mutant kernels having a glassy, near-brittle appearance. The inbreds Mo17, Oh43, N25, and A636 show good expression, with mutant kernels that are sugary and slightly wrinkled. The inbreds M14, W22, B37, A632, B76, and H99 show fair expression, with smooth, slightly translucent mutant kernels. Mutant kernels can barely be distinguished in crosses to Tama Flint, having a weakly translucent, frosted appearance. We anticipate that after Ae-5180 has been backcrossed into these inbreds for several generations, the above phenotypes will be consistently expressed.

In order to further characterize Ae-5180, and to generate potentially useful stocks, we conducted linkage tests of Ae-5180 with a series of waxy translocations, and the chromosome 5 linkage markers g18 and a2. The three T5-9 waxy translocations that we selected all showed linkage with Ae-5180 (Table 2).

Table 2. Linkage data for Ae-5180 to waxy marked translocations (wxT).

Testcross: (Ae-5180 Wx / Ae wxT) X WX WX

<u>wxT</u>	<u>Ae-5180 wx</u>	<u>Ae wx</u>	<u>Ae-5180 wx</u>	<u>Ae Wx</u>	<u>Recomb.</u>
5-9c	335	288	29	67	13.4 ± 1.3
5-9 (4790)	391	325	84	131	23.1 ± 1.4
5-9a	354	324	179	165	33.7 ± 1.5

Breakpoints: 5-9c 5S.07 9L.10  
 5-9 (4790) 5L.34 9L.45  
 5-9a 5L.69 9S.17

The tightest linkage to wx ( $p = 13.4$  cM) was with T5-9c, which has a breakpoint in the short arm of chromosome 5 close to



the centromere. This is not surprising, since ae is on the long arm of chromosome 5, about 15 cM from the centromere. We might have expected even closer linkage of Ae-5180 with wx T5-9 (4790), which has a breakpoint at 5L.34, especially since ae had been previously shown by J. N. Jenkins (Ph.D. thesis, Purdue, 1960) to be very close to the T4-5c breakpoint at 5L.27. Instead we found linkage of  $p = 23.1$  cM. Such a high percent recombination with waxy could be at least partially explained by the chromosome 9 breakpoint of wx T5-9 (4790), 9L.45, being at least 10 cM, and perhaps as many as 26 cM, from the waxy locus on the chromosome 9 linkage map. Crossovers occurring in the region between wx and the chromosome 9 breakpoint would increase the apparent linkage distance between ae and wx in this particular translocation. It is frequently difficult to correlate linkage maps with cytological maps with any degree of precision. The important point to note is that Ae-5180 does indeed show linkage with translocations involving chromosome 5. One other point of interest is that the parental and crossover classes in the wx translocation linkage data are not well-balanced. There seems to be about an equal number of plump (Ae) and sugary (Ae-5180) kernels in each set of translocation data, but for each translocation there seems to be an excess of Wx kernels.

Our linkage data for Ae-5180 and gl8 ( $12.5 \pm 0.8$  cM, Table 3) are in close agreement with the value of 11 cM reported on the 1988 linkage map for recessive ae (Maize Genetics Cooperation Newsletter, 62:142, 1988). There appears to be an abundance of

starchy (Ae) kernels over sugary (Ae-5180) kernels, but this is due to the lower percent germination of the sugary kernels when we were seedling-testing for g18. The linkage data for a2 and Ae-5180 (Table 4) yield a value ( $13.1 \pm 0.7$  cM) that is somewhat less than the reported value for a2 to ae of 22 cM, but is in agreement with a two-point test made by Jenkins, who found a value of 14 cM for a2 to ae in a backcross test.

Table 3. Linkage data for Ae-5180 to g18.

Testcross: (Ae-5180 G18 / Ae g18) X Ae Ae g18 g18

<u>Reg</u>	<u>Genotype</u>	<u>No.</u>	<u>Totals</u>
0	<u>Ae-5180 +</u>	700	
	+ <u>g18</u>	973	1673
1	<u>Ae-5180 g18</u>	103	
	+ +	136	239

% Recombination Ae-5180 -- g18 =  $12.5 \pm 0.8$

Table 4. Linkage data for a2 to Ae-5180

Testcross: a2 a2 Ae Ae x (a2 Ae-5180/A2 Ae)

<u>Reg</u>	<u>Genotype</u>	<u>No.</u>	<u>Totals</u>
0	<u>a2 Ae-5180</u>	899	
	+ +	1028	1927
1	<u>a2 +</u>	155	
	+ <u>Ae-5180</u>	135	290

% Recombination a2 -- Ae-5180 =  $13.1 \pm 0.7$

We have crossed Ae-5180 into homozygous wx stocks in order to observe whether Ae-5180 shows the same phenotypic interaction with waxy as does the standard ae allele. Kernels that are homozygous for the standard ae allele and homozygous for wx are smaller, more translucent, and have finer wrinkling than kernels that are homozygous for ae in a Wx background. The latter kernels are translucent and slightly wrinkled, but not as extremely so as those in a wx background. This effect is very striking, and requires the ae allele to be homozygous (ae ae ae) in the endosperm. If only one or two doses of ae are present, the kernels will be full and plump in either a wx or Wx background (It has been reported that in certain wx lines, it is possible to distinguish a dosage effect for ae, but we have not observed this in our lines.) Ae-5180, on the other hand, can produce the same effect in only one dose, Ae-5180 Ae Ae wx wx wx kernels (where Ae represents the wildtype starchy allele of amylose-extender) are uniformly small, translucent, and finely wrinkled. Ae-5180 Ae Ae Wx Wx Wx kernels, on the other hand, are usually just slightly smaller in size than wildtype starchy kernels, and show the wider phenotypic range from slightly translucent to wrinkled-sugary to brittle. Thus, in both wx and Wx backgrounds, one dose of Ae-5180 in the endosperm appears to have the same phenotypic effect as 3 doses of the standard ae allele.

An analogous effect can be observed when the Ae-5180 and standard ae alleles are crossed into a homozygous sul background.

It has been reported (Kramer, H. H., Whistler, R. L. and Anderson, E. G., 1956. A new gene interaction in the endosperm of maize. *Agronomy Journal* 48:170-172) that ae and sul have an interaction such that kernels homozygous for both mutants are plump, but highly translucent. This unique phenotype is different from that of either homozygous sul (wrinkled-sugary) or homozygous ae (slightly translucent, slightly wrinkled). We have produced plants that are homozygous sul, but heterozygous for Ae-5180, and outcrossed them to homozygous sul testers. The resulting ears segregate 1:1 for wrinkled-sugary kernels (Ae Ae Ae sul sul sul) and plump, translucent kernels (Ae-5180 Ae Ae sul sul sul). Thus, in a homozygous sul background, one dose of Ae-5180 in the endosperm seems to have the same phenotypic effect as 3 doses of the standard ae allele. It should be noted that the phenotypic interactions described for Ae-5180 with wx and sul are based on the physical appearance of the kernels, and have not been studied yet biochemically.

In a homozygous wx background, pollen that carries the ae allele can be readily distinguished from pollen that carries the wildtype Ae allele by a differential iodine staining technique (Moore, C. W. and Creech, R. G., 1972. Genetic fine structure analysis of the amylose-extender locus in Zea mays L. *Genetics* 70:611-619). This technique relies on overstaining the pollen grains with iodine, which is taken up by amylose but not amylopectin, followed by heat destaining. The Ae wx pollen grains, which contain amylopectin but no amylose, stain red. The

ae wx pollen grains, which contain starch that has at least some characteristics of amylose, stain dark blue. Using this technique, we found that pollen from Ae-5180 Ae wx wx plants had both dark blue and red staining pollen grains in a 1:1 ratio. Thus it seems that Ae-5180 wx pollen also stains dark blue, opening up opportunities to use differential staining to study reversion rates of Ae-5180 and to do fine-structure mapping of Ae-5180 with respect to other ae alleles.

As mentioned previously, Ae-5180 frequently shows a reduced frequency of male transmission in competition with the wildtype Ae allele. In order to study this phenomenon further, we utilized several generations of reciprocal outcrosses of heterozygous Ae-5180 Ae plants to standard starchy lines (Ae Ae). All reciprocal crosses were made during the summer of 1987. For the purposes of this discussion, the term "sugary" will be used to describe kernels showing the Ae-5180 phenotype. In all generations, Ae-5180 was fully female transmissible (Tables 5, 6, and 7). However, male transmission varied from generation to generation, and among sibling plants. The data in Table 5 present kernel counts from female and male outcross ears of reciprocally crossed plants grown from sugary kernels of the first generation male outcross ear. None of the male outcross ears showed a transmission of Ae-5180 as low as that of the original outcross ear presented in Table 1 (32.4% mutant kernels), and several ears showed normal transmission.



Table 5. Counts of starchy (Ae) and sugary (Ae-5180) kernels on exact reciprocal crosses to standard of plants grown from sugary kernels of the first generation male outcross of Ae-5180 to standard.

Plant No.	Female Outcross Ears			Male Outcross Ears		
	Starchy	Sugary	% Su	Starchy	Sugary	% Su
6093-2	161	180	52.8	213	171	44.5*
-3	256	264	50.8	345	225	39.5***
-5	195	165	45.8	235	170	42.0**
-7	216	216	50.0	259	200	43.6**
-8	204	186	47.7	176	194	52.4
6094-2	221	263	54.3	226	144	38.9***
-4	207	245	54.2	216	190	46.8
-6	272	259	48.8	202	191	48.6
-9	237	243	50.6	312	220	41.4***
-10	214	210	49.5	216	215	49.9
TOTALS	2183	2231	50.5	2400	1920	44.4***

Homogeneity  
Chi-square = 11.26  
(df = 9, N. S.)

Homogeneity  
Chi-square = 31.87  
(df = 9, p<0.001)

Symbols: \* chi-square for 1:1 significant at p<0.05  
 \*\* chi-square for 1:1 significant at p<0.01  
 \*\*\* chi-square for 1:1 significant at p<0.001

Table 6. Counts of kernels on exact reciprocal crosses to standard of plants grown from sugary kernels of second generation male outcrosses of Ae-5180 to standard.

Plant No.	Female Outcross Ears			Male Outcross Ears		
	Starchy	Sugary	% Su	Starchy	Sugary	% Su
6097-2	308	301	49.4	241	207	46.2
-7	214	214	50.0	231	214	48.1
-8	202	161	44.4*	246	236	48.9
-9	250	273	52.2	212	287	46.9
-11	281	274	49.4	300	282	48.5
6098-1	248	287	53.6	206	196	48.8
-3	207	248	54.5	288	265	47.9
-4	213	226	51.5	264	216	45.0*
-5	284	277	49.4	333	247	42.6***
-8	297	313	51.3	229	190	45.3
TOTALS	2504	2574	50.7	2550	2239	46.8***

Homogeneity  
Chi-square = 12.28  
(df = 9, N. S.)

Homogeneity  
Chi-square = 7.83  
(df = 9, N. S.)

Symbols: See Table 5

Table 7. Counts of kernels on exact reciprocal crosses to standard of plants grown from sugary kernels of third generation male outcrosses of Ae-5180 to standard.

Plant No.	Female Outcross Ears			Male Outcross Ears		
	Starchy	Sugary	% Su	Starchy	Sugary	% Su
6101-3	138	116	45.7	380	289	43.2***
-4	185	187	50.3	368	290	44.1**
-5	159	173	52.1	352	226	39.1***
-6	144	140	49.3	264	238	47.4
-11	201	196	49.4	184	144	43.9*
6102-2	229	220	49.0	302	186	38.1***
-5	167	179	51.7	200	178	47.1
-9	172	212	55.2*	401	192	32.4***
-10	272	291	51.7	381	291	43.3***
-11	218	215	49.7	257	237	48.0
TOTALS	1885	1929	50.6	3089	2271	42.4***

Homogeneity  
Chi-square = 7.58

Homogeneity  
Chi-square = 46.95

(df = 9, N. S.)

(df = 9,  $p < 0.001$ )

Symbols: See Table 5.

Table 6 presents kernel counts from reciprocal crosses to standard of plants grown from sugary kernels of second generation male outcross ears. The kernels which were planted to produce family 6097 came from an ear which had 30.8% sugary kernels, and the kernels planted to produce family 6098 came from an ear that had 29.1% sugary kernels. Thus, we expected the plants in families 6097 and 6098 to inherit the system responsible for low male transmission of Ae-5180. As can be seen from the data presented in Table 6, only 2 of 10 plants showed significantly reduced male transmission of Ae-5180.

Table 7 presents kernel counts from reciprocal crosses to standard of plants grown from sugary kernels of third generation male outcross ears. The kernels planted to produce family 6101 came from an ear with 45.6% sugary kernels. The kernels planted to produce family 6102 came from an ear with 45.8% sugary kernels. Most of the male outcross ears of families 6101 and 6102 showed reduced transmission of Ae-5180, with percentages of sugary kernels comparable to those reported in Table 5. There seems to be no pattern to the reduction in male transmission of Ae-5180 other than that it recurs even after several generations of outcrossing. Some workers (Jenkins, and others) have noted transmission anomalies associated with the standard ae allele. Perhaps there is some inherent biochemical effect of mutant amylose-extender alleles on the ability of mutant pollen to

compete with wildtype Ae pollen. The degree to which the competition of mutant pollen is affected might be influenced by genetic background and/or environment. This would not be without precedent, since wx pollen grains have been observed to germinate more slowly than Wx pollen grains in certain genetic backgrounds, resulting in a lowered frequency of wx transmission. The standard starchy lines that we use in our outcross studies have as their genetic background four different inbred lines. Thus, the individual plants in our male transmission studies are fairly heterogeneous. This could account for the wide variation in male transmission that we observe. As mentioned previously, we are in the process of moving Ae-5180 into several different inbred backgrounds in order to control genetic variation in these experiments.

In order to be certain that the reduced male transmission observed in our experiments is a real effect, and not due to poor penetrance of Ae-5180 in some of the heterozygous kernels in the male outcrosses, 100 phenotypically starchy kernels from second-generation male outcross ears were planted, and the resulting plants selfed. Out of 76 ears obtained, none segregated for sugary kernels. Therefore, the reduced number of sugary kernels in male outcross ears is due to a real reduction in male transmission of Ae-5180, and not due to poor expression of the gene. Note: Because this effect has its basis in competition and is not due to some variability in the expression of the Ae-5180 allele, it will not affect the usefulness of this unique mutant.

Summarizing, in all respects, Ae-5180 behaves as a dominant mutant allele of amylose-extender.

#### PRACTICE OF INVENTION

The transposable element-induced, dominant ae allele Ae-5180, described above, has been transferred to a larger population of seeds as follows: A collection (25 packets with 25 seeds per packet) of Code No. Ae-5180/Stand B70 has been deposited\* with the American Type Culture Collection, Rockville, Maryland. Viability of the deposited seeds was confirmed, and the seeds were assigned ATCC Accession No. 40499. These seeds all carry the dominant mutant allele of amylose-extender, Ae-5180. By using plants grown from these kernels in crosses, Ae-5180 can be transferred into any genetic background desired.

The present invention extends to methods for producing inbred and hybrid maize seeds with the dominant amylose-extender allele, Ae-5180 corresponding to the Ae-5180 mutant or the ATCC deposited seeds (Accession No. 40499). Such seeds can be used for growing high-amylose starch maize and for producing high-amylose maize seeds in bulk.

\* on 28 September 1988





Inbred Ae-5180 Line Production: The production of inbred Ae-5180 lines will be accomplished by crossing an Ae-5180 stock to the selected inbreds and repeatedly backcrossing to the inbred lines for 6 or 7 (or more) generations. Kernels carrying Ae-5180 should be selected for planting each generation. After the last backcross, kernels carrying Ae-5180 should be planted and the resulting plants self-pollinated. Ae-5180 kernels selected from the self-pollinated ears should be sown and the resulting plants self-pollinated. One third of the resulting ears will be homozygous for the Ae-5180 allele and thus would be the desired Ae-5180 conversion inbred line.

Hybrid Ae-5180 Line Production: Once Ae-5180 versions of inbred lines are produced, standard breeding methods for generating hybrid seed can be utilized. (Note: Due to the fact that Ae-5180 is a dominant, the reduction in yield of high amylose kernels that results from contamination from field corn in the present day high amylose production field utilizing the recessive ae alleles, will not occur.)

A unique production method for high amylose seeds using Ae-5180: Because Ae-5180 is dominant and is responsible for amylose levels of 70% in one or two doses in the endosperm, it will be possible to use Ae-5180 inbred or hybrid lines as male parents and high yielding (elite) Ae Ae hybrids as female parents in a production field in which the hybrid parents are either male sterile or detasseled. Although this production method is more expensive than the previous production method using hybrid Ae-5180 lines, the potential elevated yield of high amylose seeds may more than

offset these additional costs. Both male and female parents can serve as a source of high amylose seeds.

As is now thus appreciated the dominant gene from the mutant can readily transfer to any variety of maize with which it is crossed. The mutant gene can therefore be transferred into hybrid varieties of maize. Additionally, in another aspect, the invention is also to any plant and/or seed which contains the dominant gene which is produced by crossing a maize plant containing the gene with a variety to which it is to be transferred.

The claims defining the invention are as follows:

1. High amylose starch maize seeds containing at the *amylose-extender* (ae) locus at least one dominant mutant allele Ae-5180.
2. High amylose maize seeds produced by crossing two maize lines, said seeds containing at the *amylose-extender* (ae) locus at least one dominant mutant allele Ae-5180.
3. High amylose starch maize seeds containing at the *amylose-extender* (ae) locus homozygous Ae-5180 alleles.
4. The maize seeds of claim 3, which are hybrid maize seeds.
5. The maize seeds of claim 3, which are inbred maize seeds.
6. Maize plants grown from the maize seeds of any one of claims 1 to 5.
7. Viable maize seeds and succeeding generations thereof grown from the seeds deposited under ATCC Accession No. 40499, and maize seeds to which the Ae-5180 dominant mutant allele of said deposited seeds has been transferred and succeeding generations thereof, said maize seeds and succeeding generations thereof containing the dominant mutant allele Ae-5180 at their *amylose-extender* (ae) locus.
8. The method of developing hybrid maize seeds containing high amylose starch, comprising crossing a first maize line with a second maize line, at least one of said lines containing at its *amylose-extender* (ae) locus at least one dominant mutant allele Ae-5180.
9. The method of claim 8, wherein at least one of said lines contains at the *amylose-extender* (ae) locus homozygous Ae-5180 alleles.
10. The method of claim 8, wherein both of said lines contain at the *amylose-extender* (ae) locus homozygous Ae-5180 alleles.
11. The method for directly producing high amylose maize seeds, comprising crossing a homozygous Ae-5180 line as a male onto a detasseled or male sterile wild type (Ae Ae) hybrid maize line.

DATED this 18th day of March 1992.

IOWA STATE UNIVERSITY RESEARCH FOUNDATION, INC.

By their Patent & Trade Mark Attorneys:

CALLINAN LAWRIE



*Michael J. Houlihan.*